

WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule comprising a nucleotide sequence encoding at least one modified intein, a functional derivative or homolog thereof, wherein said nucleotide sequence further comprises one or more recombination sites.
2. An isolated nucleic acid molecule comprising: (a) a nucleotide sequence encoding at least one modified intein, a functional derivative or homolog thereof, wherein said nucleotide sequence further comprises one or more recombination sites; and (b) one or more topoisomerase recognition sites and/or one or more topoisomerases.
3. An isolated nucleic acid molecule comprising a nucleotide sequence encoding at least one modified intein, a functional derivative or homolog thereof, wherein said nucleotide sequence further comprises one or more recombination sites.
4. An isolated nucleic acid molecule comprising: (a) a nucleotide sequence encoding at least one modified intein, a functional derivative or homolog thereof, wherein said nucleotide sequence further comprises one or more recombination sites; and (b) one or more topoisomerase recognition sites and/or one or more topoisomerases.
5. The nucleic acid molecule of claim 1 or 3, wherein said nucleic acid molecule is a linear or circular molecule.
6. The nucleic acid molecule of claim 1 or 3, wherein said nucleic acid molecule comprises two or more recombination sites.
7. The nucleic acid molecule of claim 1, 2, 3, or 4, wherein one recombination site is flanked on one end by a cloning site.
8. The nucleic acid molecule of claim 7, wherein said cloning site contains one or more one unique restriction enzyme sites

9. The nucleic acid molecule of claim 6, wherein at least one of said two or more recombination sites flanks one of the ends of a topoisomerase recognition site in said molecule.

10. The nucleic acid molecule of claim 1 or 3, wherein said recombination sites are selected from the group consisting of:

- (a) *attB* sites,
- (b) *attP* sites,
- (c) *attL* sites,
- (d) *attR* sites,
- (e) *lox* sites,
- (f) *psi* sites,
- (g) *dif* sites,
- (h) *cer* sites,
- (i) *frt* sites,

and mutants, variants, and derivatives of the recombination sites of (a), (b), (c), (d), (e), (f), (g), (h) or (i) which retain the ability to undergo recombination.

11. The nucleic acid molecule of claim 1 or 3, wherein said topoisomerase recognition site is recognized and bound by a type I topoisomerase.

12. The nucleic acid molecule of claim 11, wherein said type I topoisomerase is a type IB topoisomerase.

13. The nucleic acid molecule of claim 12, wherein said type IB topoisomerase is selected from the group consisting of eukaryotic nuclear type I topoisomerase and a poxvirus topoisomerase.

14. The nucleic acid molecule of claim 13, wherein said poxvirus topoisomerase is produced by or isolated from a virus selected from the group consisting of vaccinia virus, Shope fibroma virus, ORF virus, fowlpox virus, molluscum contagiosum virus and *Amsacta moorei* entomopoxvirus.

15. The nucleic acid molecule of claim 1 or 3, wherein said nucleic acid molecule further comprises a nucleotide sequence encoding a selectable marker.

16. The nucleic acid molecule of claim 1 or 3, wherein said nucleic acid molecule further contains a prokaryotic or eukaryotic origin of replication.

17. The nucleic acid molecule of claim 1, wherein the nucleotide sequence encoding the at least one modified intein is operably linked to a sequence which is capable of regulating transcription.

18. The nucleic acid molecule of claim 17, wherein the sequence which is capable of regulating transcription is selected from the group consisting of:

- (a) a promoter;
- (b) an enhancer; and
- (c) a repressor.

19. The nucleic acid molecule of claim 18, wherein the promoter is either an inducible promoter or a constitutive promoter.

20. A vector comprising the nucleic acid molecule of claim 1 or 3.

21. The vector of claim 20, wherein said vector is an expression vector.

22. The expression vector of claim 21, wherein the expression vector is selected from the group consisting of pcDNAGW-DT(sc), Pentr-DT(sc), pcDNA-DEST41, Pentr/D-TOPO, Pentr/SD/D-TOPO, pcDNA3.2/V5/GWD-TOPO and pcDNA6.2/V5/GWD-TOPO.

23. A host cell comprising the isolated nucleic acid molecule of claim 1.

24. A host cell comprising the vector of claim 21.

25. A host cell comprising the vector of claim 22.

26. A method of cloning at least one hybrid nucleic acid molecule comprising:

(a) providing at least a first population of nucleic acid molecules comprising at least one modified intein nucleotide sequence or a functional derivative or homolog thereof wherein all or a portion of such molecules contain at least a first and a second recombination site;

(b) providing at least a second population of nucleic acid molecules encoding at least one negative selectable marker located between a third and a fourth recombination site, and wherein either the first or the second recombination site of the first population of nucleic acid molecules is capable of recombining with either the third or the fourth recombination site of the second population of nucleic acid molecules;

(c) conducting a recombination reaction such that all or a portion of the molecules in the first population comprising at least one modified intein nucleotide sequence or a functional derivative or homolog thereof is recombined with one or more molecules from the second population to form a third population of hybrid nucleic acid molecules;

(d) cloning the third population of hybrid nucleic acid molecules; and

(e) introducing the cloned hybrid nucleic acid molecules into a suitable host cell.

27. A method of cloning at least one hybrid nucleic acid molecule comprising:

(a) providing a first population of first nucleic acid segments comprising at least one modified intein nucleotide sequence or a functional derivative or homolog thereof, wherein the first nucleic acid segment contains a first and a second recombination site;

(b) providing a second population of nucleic acid molecules comprising a vector molecule containing a nucleic acid molecule encoding at least one negative selectable marker, wherein the selectable marker is located between a third and a fourth recombination site, wherein either the first or the second recombination site of the first nucleic acid segment is capable of recombining with either the third or the fourth recombination site of the second population of nucleic acid molecules;

(c) conducting a recombination reaction such that all or a portion of the first nucleic acid segments comprising at least one modified mutant intein nucleotide sequence or a functional derivative or homolog thereof are recombined with all or a portion of the vector molecules comprising at least one negative selectable marker to form a third population of hybrid nucleic acid molecules;

(d) cloning the third population of hybrid nucleic acid molecules; and

(e) introducing the cloned hybrid nucleic acid molecules into a suitable host cell.

28. The method of claim 26 or 27, wherein the recombination sites comprise one or more recombination sites selected from the group consisting of:

- (a) *lox* sites;
- (b) *psi* sites;
- (c) *dif* sites;
- (d) *cer* sites;
- (e) *frt* sites;
- (f) *att* sites; and
- (g) mutants, variants, and derivatives of the recombination sites of (a), (b), (c), (d), (e), or (f) which retain the ability to undergo recombination.

29. The method of claim 26 or 27, wherein the recombination is caused by mixing the first population of nucleic acid molecules and the second population of nucleic acid molecules with one or more recombination proteins under conditions which favor the recombination.

30. The method of claim 29, wherein the recombination proteins comprise one or more proteins selected from the group consisting of:

- (a) Cre;
- (b) Int;
- (c) IHF;
- (d) Xis;
- (e) Fis;
- (f) Hin;

- (g) Gin;
- (h) Cin;
- (i) Tn3 resolvase;
- (j) TndX;
- (k) XerC; and
- (l) XerD.

31. The method of claim 26 or 27, further comprising selecting for the population of hybrid nucleic acid molecules.

32. The method of claim 31, further comprising selecting for the population of hybrid nucleic acid molecules and against the first population of nucleic acid molecules.

33. The method of claim 32, further comprising selecting against cointegrate molecules and byproduct molecules such that insertion of the first nucleic acid molecules and resolving of the resultant cointegrate results in excision or loss of the negative selectable marker and retention of the at least one intein nucleotide sequence or a functional derivative or homolog thereof.

34. The method of claim 26 or 27, wherein at least one of the nucleic acid molecules is operably linked to a sequence which is capable of regulating transcription.

35. The method of claim 34, wherein the sequence which is capable of regulating transcription is selected from the group consisting of:

- (a) a promoter;
- (b) an enhancer; and
- (c) a repressor.

36. The method of claim 35, wherein the promoter is either an inducible promoter or a constitutive promoter.

37. The method of claim 26 or 27, wherein one or more of the nucleic acid molecules further encodes a selectable marker.

38. The method of claim 26 or 27, wherein one or more of the nucleic acid molecules contains a prokaryotic or eukaryotic origin of replication.

39. The method of claim 26 or 27, wherein translation of an RNA produced from the cloned nucleic acid molecules results in the production of a fusion protein.

40. The method of claim 26 or 27, wherein the second population of nucleic acid molecules further encodes a fusion protein.

41. The method of claim 26 or 27, wherein the second population of nucleic acid molecules further encodes a polypeptide selected from the group consisting of:

- (a) the Fc portion of an immunoglobulin;
- (b) β -glucuronidase;
- (c) a fluorescent protein;
- (d) a purification tag; and
- (e) an epitope tag.

42. The method of claim 41, wherein the second population of nucleic acid molecules further encodes a fluorescent protein selected from the group consisting of:

- (a) green fluorescent protein;
- (b) yellow fluorescent protein;
- (c) red fluorescent protein; and
- (d) cyan fluorescent protein.

43. The method of claim 41, wherein the second population of nucleic acid molecules further encodes an additional purification tag selected from the group consisting of:

- (a) an epitope tag;

- (b) maltose binding protein;
- (c) a six histidine tag; and
- (d) glutathione S-transferase.

44. The method of claim 26 or 27, wherein at least one of the nucleic acid molecules encodes all of part of an open reading frame and at least one of the nucleic acid molecules contains a sequence which is capable of regulating transcription.

45. The method of claim 26 or 27, wherein the nucleic acid molecules comprise nucleic acid molecules of one or more libraries.

46. The method of claim 45, wherein the one or more libraries comprise either Cdna or genomic DNA.

47. The method of claim 46, wherein the one or more libraries comprise nucleic acid molecules which encode variable domains of antibody molecules.

48. The method of claim 47, wherein the one or more libraries comprise nucleic acid molecules which encode variable domains of antibody light and heavy chains.

49. A composition comprising the population of hybrid nucleic acid molecules produced by the method of claim 26 or 27.

50. A population of recombinant host cells comprising the population of hybrid nucleic acid molecules produced by the method of claim 26 or 27.

51. A kit for cloning, joining, deleting, or replacing nucleic acid segments, the kit comprising (1) one or more recombination proteins or a composition comprising one or more recombination proteins, (2) at least one nucleic acid molecule comprising one or more recombination sites having at least two different recombination specificities, and (3) one or more components selected from the group consisting of:

- (a) nucleic acid molecules comprising additional recombination sites;
- (b) one or more enzymes having ligase activity;

- activity;
- (c) one or more enzymes having polymerase activity;
 - (d) one or more enzymes having reverse transcriptase activity;
 - (e) one or more enzymes having restriction endonuclease activity;
 - (f) one or more primers;
 - (g) one or more nucleic acid libraries;
 - (h) one or more supports;
 - (i) one or more buffers;
 - (j) one or more detergents or solutions containing detergents;
 - (k) one or more nucleotides;
 - (l) one or more terminating agents;
 - (m) one or more transfection reagents;
 - (n) one or more host cells; and
 - (o) instructions for using the kit components.

52. The kit of claim 51, wherein the recombination sites having at least three different recombination specificities each comprising *att* sites with different seven base pair overlap regions.

53. The kit of claim 51, wherein the composition comprising one or more recombination proteins is capable of catalyzing recombination between *att* sites.

54. The kit of claim 51, wherein the composition comprising one or more recombination proteins capable of catalyzing a BP reaction, an LR reaction, or both BP and LR reactions.